AmoyDx® Tissue DNA Kit

(Spin Column)

For purification of DNA from human tissue or pleural effusion precipitation

Instruction for Use

For Research Use Only

REF 8.02.24301X036G 36 tests

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Intended Use

The AmoyDx® Tissue DNA Kit is specially designed for isolation and purification of DNA from human tissue or pleural effusion precipitation. The purified DNA is suitable for the downstream PCR amplification, genotype analysis, restriction enzyme digestion and other experiments.

Principles of the Procedure

This kit uses efficient tissue lysis buffer system, combined with silicone membrane adsorption column technology, isolate and purify the genomic DNA from human tissue or pleural effusion precipitation.

Kit Contents

This kit contains sufficient reagents to perform 36 tests (Table 1).

Table 1 Kit Contents

Component	Quantity	Tube No.
DNA Spin Columns	36 tubes	—
Collection Tubes (2 mL)	72 tubes	—
Centrifugal Tubes (1.5 mL)	72 tubes	—
Buffer DTL	10 mL/vial ×1	1
Proteinase K Solution	900 μL/tube ×1	2
Buffer DTB	10 mL/vial ×1	3
Buffer DW1 (concentrate)	13 mL/vial ×1	4
Buffer DW2 (concentrate)	6 mL/vial ×1	5
Buffer DTE	8 mL/vial ×1	6

Note:

1) ***Buffer DTB*** *and* ***Buffer DW1*** *contain guanidine salt, not compatible with disinfectants containing bleach or acidic solutions.*
2) *For the first time use, add 17 mL ethanol (96~100%) into* ***Buffer DW1 (concentrate)*** *and mix thoroughly; add 24 mL ethanol (96~100%) into* ***Buffer DW2 (concentrate)*** *and mix thoroughly . Tick the check box on the bottle label.*

Storage and Stability

The shelf life of the kit is 12 months. The kit should be stored dry at room temperature (10~30℃).

Additional Reagents and Equipment Required but Not Supplied

1) Ethanol (96~100%).
2) Physiological saline.
3) Water bath or heated orbital incubator (56℃ adjustable).
4) Microcentrifuge (10000~12000 ×g adjustable).
5) Vortexer.
6) Palm centrifuge.
7) Sterile, DNase-free pipet tips.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.

- DO NOT use any other reagent in the other test kits.

Safety Information

- ***Buffer DTB*** *and* ***Buffer DW1*** contain guanidine salt, which can form highly reactive compounds when combined with bleach. **Do not add bleach or acidic solutions directly to the sample-preparation waste.** If the liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.
- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- If a spill contains potentially infectious reagents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent cross-contamination.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Starting material

Rich DNA enzymes present in tissue. In the process of grinding and nucleic acid separation, DNA is readily biodegradable. Before using this kit to do DNA separation, be sure to:

1) Removed fresh tissue sample should be immediately stored in tissue preservation solution at -70℃. Without -70℃, it can be stored at -20℃ with no longer than a month.
2) Tissue sample's storage time should be less than three years at -70℃.
3) For the tissue samples which have been stored in liquid nitrogen or at -70℃, please add 10-fold volume of tissue protective solution before isolating, and thaw at 4℃.

Assay Procedure

1. Sample pretreatment

1.1 Pleural fluid samples:

1.1.1 Take 10~40 mL of pleural fluid samples, centrifuge at 3000 ×g for 10 min and remove the supernatant by pipetting.

1.1.2 Add 1-2mL physiological saline, and mix by vortexing; centrifuge at 3000 ×g for 10 min and remove the supernatant by pipetting.

1.1.3 Place 20~80 mg precipitation in a clean 1.5 mL centrifugal tube, add 180 μL Buffer DTL and treat the precipitate as homogenized one.

1.2 Human tissue samples:

1.2.1 Add 1mL physiological saline into 20~60 mg tissue samples (splenic tissue samples: 5~10 mg), and mix by vortexing; centrifuge at 12000 ×g for 2 min and remove the supernatant by pipetting.

1.2.2 Add 180 μL Buffer DTL and treat the tissue as homogenized precipitate or cut into tissue blocks which are less than 1 mm^3

2. DNA Extraction

Note:

- *For the first time use, please add 17 mL ethanol (96~100%) into* ***Buffer DW1 (concentrate)****, add 24 mL ethanol (96~100%) into*

__Buffer DW2 (concentrate)__, and mark it clearly.

- *Before the DNA extraction, please check the reagents without leakage. Shake the reagents gently to mix the solutions. If the reagents contain precipitates, dissolved by heating at 50 ℃.*

2.1 Add 20 μL **Proteinase K Solution**, mix by vortexing.

2.2 Incubate at 56℃ to obtain more perfectly clear tissue lysate for 1-4 hours; Different samples take different period of time. If the tissue has not been completely lysed, or need higher concentration of DNA, incubate for further time or overnight.

2.3 Take out the sample tube, briefly centrifuge for 5~10 seconds. If RNA-free genomic DNA is required, allow the sample to cool to room temperature, add 2 μL RNase A (100 mg/mL) and incubate for 5 min at room temperature.

2.4 Add 200 μL **Buffer DTB** and 200 μL ethanol (96~100%), mix by vortexing.

2.5 Briefly centrifuge for 5~10 seconds.

2.6 Transfer the entire lysate to the DNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 8000 ×g for 1 min.

2.7 Discard the flow-through in collection tube.

2.8 Add 600 μL **Buffer DW1** to DNA Spin Column, centrifuge at 8000 ×g for 1 min.

2.9 Discard the flow-through in collection tube.

2.10 Add 600 μL **Buffer DW2** to DNA Spin Column, centrifuge at 8000 ×g for 1 min.

2.11 Discard the collection tube with flow-through.

2.12 Place the DNA Spin Column in a clean 2 mL collection tube, centrifuge at 12000 ×g for 3 min.

2.13 Discard the collection tube with flow-through.

2.14 Place the DNA Spin Column in a clean 1.5 mL centrifugal tube.

2.15 Apply 30~100 μL **Buffer DTE** to the center of the membrane. Do not touch the membrane.

2.16 Incubate at room temperature for 1~5 min.

2.17 Centrifuge at 12000 ×g for 1 min.

2.18 The eluted DNA is immediately ready for use or for storage under -20℃.

Note: Buffer DTE is only for elution and storage of DNA, NOT for other use.

Limitations

1) The quality of extracted DNA is subject to the influence of such factors as sample source, sampling process, collection site, storage conditions.
2) Sample quality has a high impact on quality and amount of the purified DNA.

Symbols

Manufacturer

Catalogue Number

Batch Code

Use By

Contains Sufficient for <n> Tests

Temperature Limitation

Consult Instructions For Use

Keep Dry

This Way Up

Fragile, Handle With Care